Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 6492–6501
Bioorganic & Medicinal Chemistry

Structure–activity-relationship studies of conformationally restricted analogs of combretastatin A-4 derived from SU5416

Bulbul Pandit,[a] Yanjun Sun,[a] Ping Chen,[a] Dan L. Sackett,[b] Zhigen Hu,[a] Wendy Rich,[a] Chenglong Li,[a] Andrew Lewis,[a] Kevin Schaefer[a] and Pui-Kai Li[a,*]

[a]*Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
[b]*Laboratory of Integrative and Medical Biophysics, National Institute of Child Health and Human Development, National Institute of Health, Bethesda, MD 20892, USA*

Received 16 March 2006; revised 31 May 2006; accepted 8 June 2006
Available online 24 July 2006

Abstract—A series of combretastatin A-4 analogs derived from the ATP competitive, VEGF receptor tyrosine kinase inhibitor, SU5416 were synthesized. The cytotoxic effects of the analogs were evaluated against PC-3 and MDA-MB-231 cancer cell lines, as well as their abilities to inhibit tubulin polymerization. Results are compared to those of compound **1**, our lead compound previously reported.

1. Introduction

Microtubules are involved in cellular functions such as cell transport, movement and separation of chromosomes during mitosis. Anti-microtubule agents are a class of anti-tumor agents that target tubulin, the building block of microtubules. Mechanistically, anti-microtubule agents can be classified into three classes: (i) microtubule stabilizing agents, (ii) vinca site binding agents and (iii) colchicine site binding agents. Of the three classes of anti-microtubule agents, colchicine site agents are the only class of agents that do not have a representative drug in clinical use for cancer. However, these agents are structurally the most diverse group among the three classes of tubulin binding agents. They include natural products as well as synthetic small molecules (Fig. 1).[1,2] Among all the colchicine site agents, combretastatin A-4 (CA-4) has received special attention in the last few years.[3,4] In addition to its potent cytotoxicity and inhibitory activity on tubulin polymerization, CA-4 is one of the few anti-microtubule agents reported to have selective vascular targeting activity.[5,6] CA-4 and its water-soluble prodrug, combretastatin A-4 phosphate (CA-4P), are selectively cytotoxic to rapidly proliferating tumor vasculature than normal blood vessels resulting in reduced blood flow to tumor and eventual hemorrhagic necrosis.[7,8] The anti-tumor and anti-vascular activities of CA-4 analogs have been demonstrated both in animal and human studies.[9–12]

Many analogs of CA-4 have been designed to study the structure–activity-relationship of the molecule in order to enhance both the cytotoxic and selective vascular targeting activity.[13–24] We have been using SU5416,[25,26] a potent ATP competitive, VEGF receptor tyrosine kinase inhibitor, as a template for the design of inhibitors for other growth factor receptors. Recently, we discovered and reported a 2-indolinone containing compound (compound **1**, Fig. 2) with potent growth inhibitory activities on hormone-independent prostate (PC-3) and breast (MDA-MB-231) cancer cell lines with IC_{50} values in low to subnanomolar range.[27] Structurally, compound **1** resembles CA-4 (Fig. 2). In this paper, we report the NCI 60 cell line screening and cell cycle analysis data of compound **1** and the synthesis and biological activities of its structurally related analogs (compounds **2**–**25**).

2. Results and discussion

2.1. Chemistry

The 6-substituted 3-benzylideneindolin-2-ones (compounds **1**–**25**) were synthesized by coupling 6-substituted

Keywords: Combretastatins; SU5416; Antimicrotubule; Antitumor agents; Cell cycle arrest; Colchicine.
* Corresponding author. Tel.: +1 614 688 0253; fax: +1 614 688 8556; e-mail: li.27@osu.edu

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.06.017

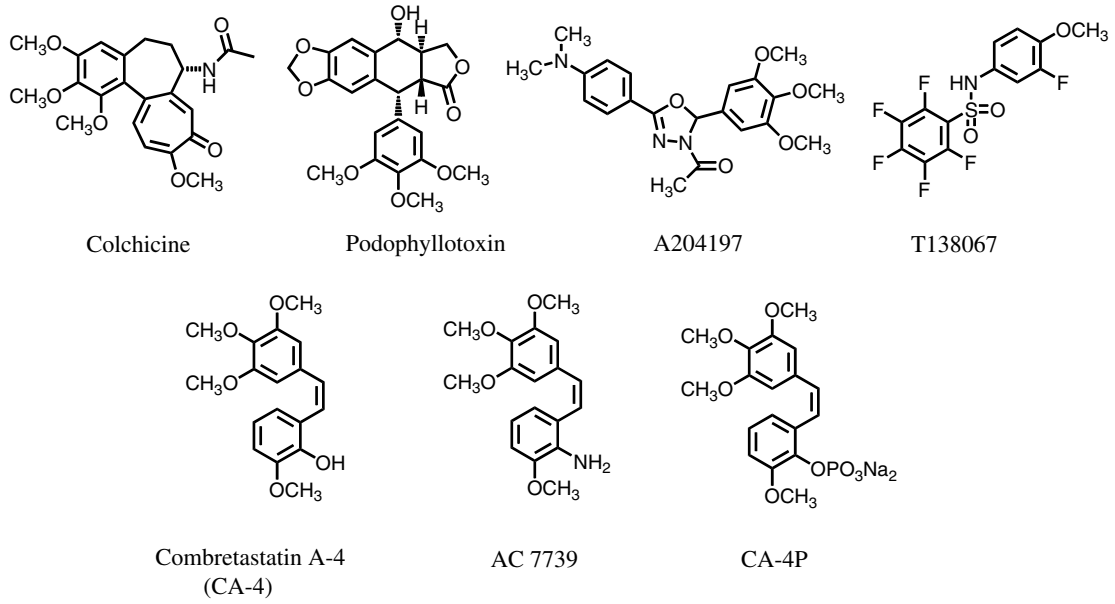


Figure 1. Examples of colchicine like agents and combretastatin A4 analogs.

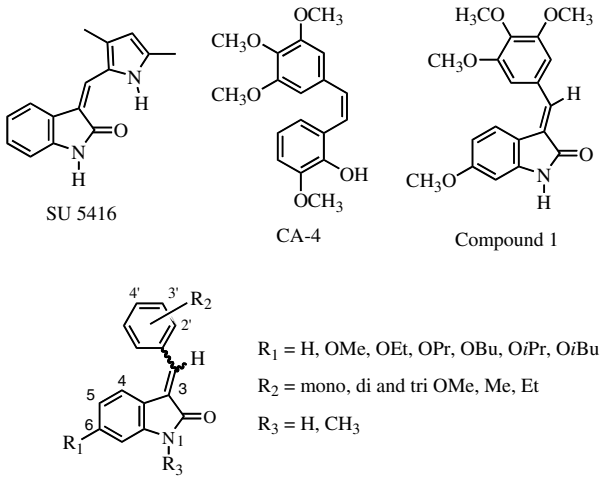


Figure 2. Structures of SU5416, CA-4, compound **1** and its analogs.

indolin-2-ones and substituted benzaldehydes in the presence of a secondary amine, such as piperidine (Fig. 3). All the 6-alkoxyindolin-2-ones were prepared according to the reported procedure.[28] Indolin-2-one and most of the substituted benzaldehydes were commercially available except for 2,4,6-triethylbenzaldehyde, which was prepared according to the reported procedure.[29]

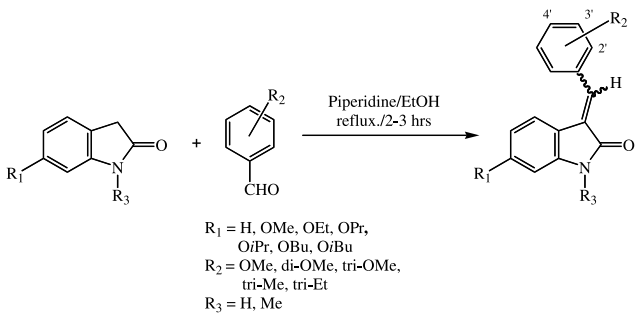


Figure 3. Synthesis of compounds **1**–**25**.

In general, the coupling reaction gave the *E* isomers as the major product, and only in three cases (compounds **2**, **5**, and **12**), *Z* isomers were also isolated. The E configurations of the compounds were assigned based upon the chemical shifts of the protons at the C-2′ and C-6′ positions in the phenyl ring at the C-3 position or 2D NOE analysis. It has been demonstrated through NOE experiment that the chemical shift of C-2′ and C-6′ protons of three substituted benzylidenindolin-2-ones is approximately 7.45–7.84 for the *E* isomers and 7.85–8.53 for the *Z* isomers.[25]

2.2. Biological activity

2.2.1. NCI cancer cell lines screen of compound 1. The anti-proliferative profile of compound **1** was evaluated at the National Cancer Institute (NCI) against 53 human cancer cell lines. The results are shown in Table 1. Compound **1** was extremely potent with GI_{50} (http://dtp.nci.nih.gov/docs/compare/compare_methodology.html) below 10 nM towards 46 out of 53 human cancer cell lines tested. The compound is active at <10 nM including all colon, CNS, prostate, and renal cancer cell lines. An interesting observation from this screen was that compound **1** was equally effective on Adriamycin resistant NCI/ADR-RES cell line.

2.2.2. Effect of compound 1 on cell cycle. The effect of compound **1** on the cell cycle progression of proliferating tumor cells was studied in a concentration-dependent cell cycle analysis. Compound **1** caused a G_2/M phase arrest in PC-3 cells following 24 h of incubation (Fig. 4). Table 2 lists the percentage of cells arrested in the G_2/M phase. Podophyllotoxin, a known anti-mitotic, anti-microtubule agent, was used as control.

Table 1. GI_{50} values on NCI cancer cell line screen of compound **1**

CELL-LINE	GI_{50} (mol/l)
Leukemia	
CCRF-CEM	$<10^{-8}$
MOLT-4	$<10^{-8}$
RPMI-8226	$<10^{-8}$
NSCLC	
A549/ATCC	$<10^{-8}$
EKVX	$<10^{-8}$
HOP-62	
HOP-92	2.5×10^{-7}
NCI-H226	$<10^{-8}$
NCI-H23	$<10^{-8}$
NCI-H322M	2.54×10^{-6}
NCI-H460	$<10^{-8}$
NCI-H522	$<10^{-8}$
Colon cancer	
COLO-205	$<10^{-8}$
HCC-2998	$<10^{-8}$
HCT-116	$<10^{-8}$
HCT-15	$<10^{-8}$
HT29	$<10^{-8}$
KM12	$<10^{-8}$
SW-260	$<10^{-8}$
CNS cancer	
SF-268	$<10^{-8}$
SF-295	$<10^{-8}$
SF-539	$<10^{-8}$
SNB-19	$<10^{-8}$
SNB-75	$<10^{-8}$
U251	$<10^{-8}$
Prostate cancer	
PC-3	$<10^{-8}$
DU-145	$<10^{-8}$
Melanoma	
LOX IMVI	$<10^{-8}$
MALME-3M	
M14	$<10^{-8}$
SK-MEL-2	1.33×10^{-5}
SK-MEL-28	$<10^{-8}$
SK-MEL-5	$<10^{-8}$
UACC-257	4.64×10^{-6}
UACC-62	$<10^{-8}$
Ovarian cancer	
IGROV1	$<10^{-8}$
OVCAR-3	$<10^{-8}$
OVCAR-4	1.4×10^{-5}
OVCAR-5	$<10^{-8}$
OVCAR-8	$<10^{-8}$
SK-OV-3	1.43×10^{-7}
Renal cancer	
786-0	$<10^{-8}$
A498	$<10^{-8}$
CAKI-1	$<10^{-8}$
RFX 393	$<10^{-8}$
SN 12C	$<10^{-8}$
TK-10	$<10^{-8}$
UO-31	$<10^{-8}$
Breast cancer	
MCF 7	$<10^{-8}$
NCI/ADR-RES	$<10^{-8}$
MDA-MB-231/ATCC	$<10^{-8}$
HS 578T	$<10^{-8}$
MDA-MB-435	$<10^{-8}$
BT-549	$<10^{-8}$
T-47D	1.03×10^{-5}

2.2.3. Anti-proliferative activities of compounds 2–25. In this study, we synthesized 24 new compounds and tested them against hormone-independent prostate (PC-3) and breast (MDA-MB-231/ATCC) cancer cells to elucidate the structure–activity-relationship of their anti-proliferative activities (Tables 3–5). In addition, selected compounds were also tested for their abilities to inhibit tubulin polymerization (Table 6).

Compound **1** contains the 3′,4′,5′-tri-OMe groups on the benzylidene moiety, a common structural feature of colchicine, CA-4, podophyllotoxin, and others. Our first structure–activity-relationship study was to investigate the substitution pattern of the tri-OMe groups in compound **1**. The location of the tri-OMe groups on the benzylidene ring plays a critical role for the compound to exhibit potent cytotoxicity. Rotating the 3′,4′,5′-tri-OMe groups in compound **1** to the 2′,3′,4′ positions decreases the cytotoxicity by three orders of magnitude (Table 3). It is interesting to note that a similar substitution pattern (2′,3′,4′-tri-OMe to replace 3′,4′,5′-tri-OMe) in a combretastatin analog also resulted in dramatic (5-fold) decrease in cytotoxicity.[30] Interestingly, transferring the 3′,4′,5′-tri-OMe groups in compound **1** to the 2′,4′,5′ (compound **4**) or 2′,4′,6′ (compound **6**) positions retained the potent cytotoxicity (Table 3).

The substitution pattern of the trimethoxy groups is not the only factor that determines the cytotoxicity of the compounds. The substituents also play a significant role. Substituting the 2′,4′,6′-tri-OMe groups in compound **6** with 2′,4′,6′-tri-Me or 2′,4′,6′-tri-Et groups results in a decrease in cytotoxicity by 73–500-fold (Table 3). This might be due to the loss of the hydrogen bonding ability or altering the electronic character of the phenyl ring in compounds **7** and **8**. In addition, the N-1 hydrogen on compound **1** is not critical for cytotoxicity. Substituting the N-1 hydrogen with a methyl group (compound **9**) retains cytotoxicity with IC_{50} values of 31 and 37 nM on PC-3 and MDA-MB-231 cells, respectively (Table 3).

Our second study was to investigate the importance of the number of methoxy groups on the benzylidene ring with respect to the cytotoxicity. This study shows that the tri-OMe group is optimum for activity. Reducing the number of OMe groups from 3 (compound **1**) to 2 (compounds **10**–**16**) reduced the cytotoxicity (Table 4). The reduction ranges from 6–500-fold in PC-3 cells and 80–3400-fold in MDA-MB-231 cells. The most dramatic reduction in cytotoxicity is observed when the di-OMe groups are at 2′ and 3′ positions (500- and 2400-fold decrease in cytotoxicity in PC-3 and MDA-MB-231 cells, respectively) (Table 4). As discussed in the previous section, compound **3**, an analog of compound **1** which also contains 2′ and 3′ OMe groups in

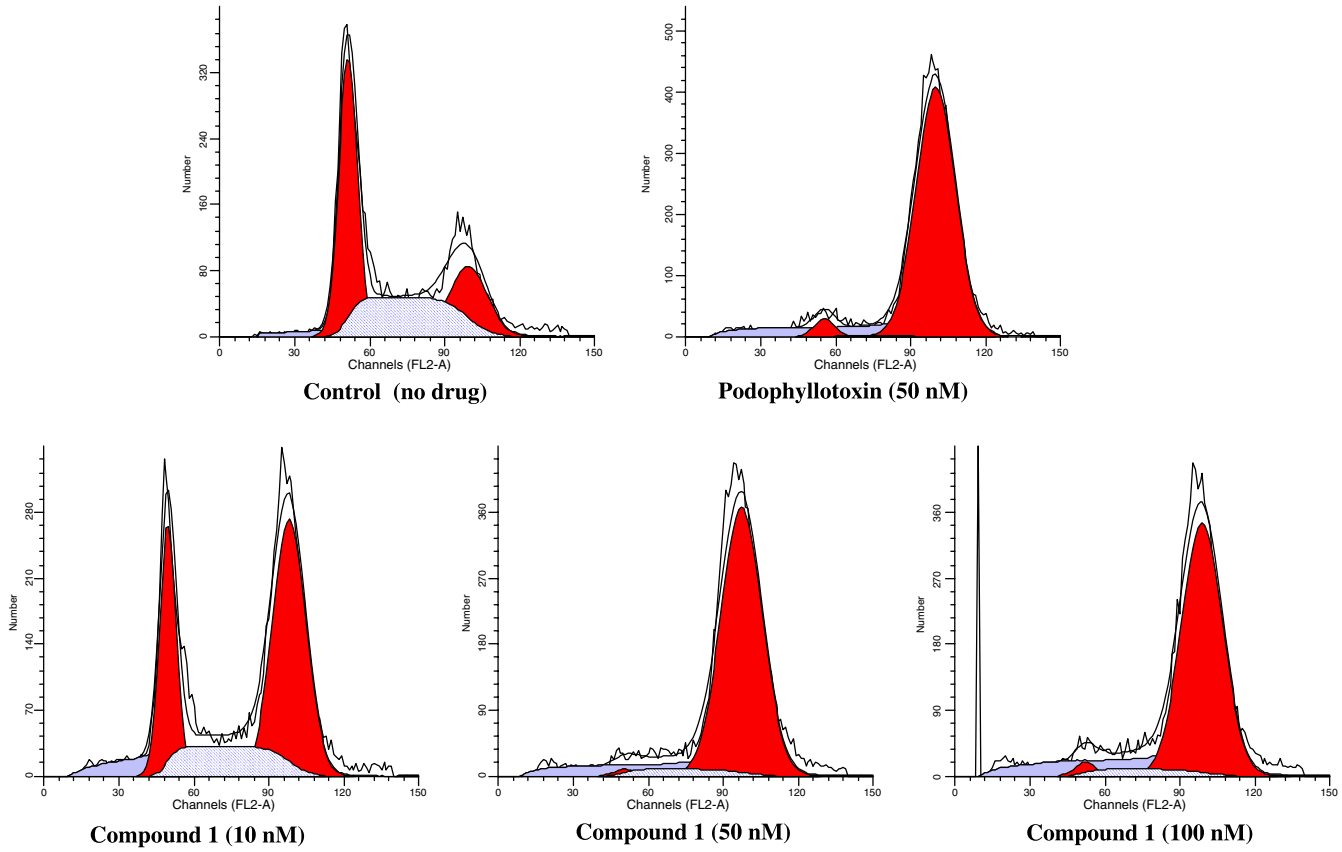


Figure 4. DNA profiles of PC-3 cells treated for 24 h with compound **1** and Podophyllotoxin.

Table 2. Percent of cells in G_2/M phase after treatment with compound **1** and podophyllotoxin for 24 h

Treated group	% G_2-M[a]
Control	12.04
Podophyllotoxin 100 nM	92.5
Compound **1** 10 nM	55.8
Compound **1** 50 nM	92.04
Compound **1** 100 nM	91.6

[a] % values are average of two separate experiments.

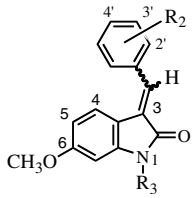
the benzylidene nucleus, exhibits a 3 orders of magnitude decrease in cytotoxicity as compared to compound **1** (Table 3). Based on these results, we postulate that the substitution of OMe groups on the 2′ and 3′ positions is detrimental to the cytotoxicity of the compounds. Reducing the number of methoxy groups from 3 to 1 (compounds **17**–**19**) also decreases the cytotoxicity of the compounds by 2–3 orders of magnitude (Table 4). The order of cytotoxicity is based on the number of OMe groups on benzylidene ring, with 3 > 2 > 1.

Next, we investigated the significance of the 6-OMe group on the 2-indolinone ring of compound **1**. Six analogs of compound **1** (compounds **20**–**25**) were synthesized with different substituents at the 6 position. The 6-OMe group is essential for potent cytotoxicity. Replacing the 6-OMe group in compound **1** with H (compound **20**) resulted in the elimination of cytotoxicity (Table 5). Our next approach was to investigate the bulk tolerance at the 6 position by substituting the OMe with alkoxy groups of varying sizes. The alkoxy groups contain both the straight chains (OEt, compound **21**; OPr, compound **22**; and OBu, compound **23**) and the branched chain analogs (O-*i*-P-r, compound **24**; O-*i*-Bu, compound **25**). In the straight chain series, replacing the 6-OMe in compound **1** with a OEt group retains the potent cytotoxicity. As the length of the alkoxy group increases, the activity decreases slightly. The extent of reduction is similar if the alkoxy group is switched from straight to branched chains.

To investigate whether the cytotoxicity of the compounds has direct correlation with their abilities to inhibit tubulin polymerization, selected compounds were evaluated for inhibition of tubulin polymerization using purified porcine tubulin (Table 6). The most potent compound in this series in tubulin polymerization is compound **1** with an IC_{50} of 4.5 μM. This is in agreement with **1** being the most cytotoxic compound. It should be noted that for the compounds with potent cytotoxicity, there is a positive correlation between the inhibition of tubulin polymerization and cytotoxicity (Table 6). In general, compounds with trimethoxy groups on the benzylidene ring exhibit the highest inhibition.

In the previous section, we discussed about the existence of *E* and *Z* isomers in some of the compounds

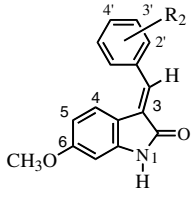
Table 3. Anti-proliferative activities of compounds **2–9**



Compound	R_2	R_3	*E* or *Z* isomer	PC-3[a] (IC_{50} nM)	MDA-MB-231[a] (IC_{50} nM)
1	3′,4′,5′-OCH_3	H	*E*	8 ± 2.3	0.9 ± 0.07
2	3′,4′,5′-OCH_3	H	*Z*	34 ± 10	4.0 ± 1.0
3	2′,3′,4′-OCH_3	H	*E*	5800 ± 60	1100 ± 140
4	2′,4′,5′-OCH_3	H	*E*	6 ± 1.5	4.0 ± 0.6
5	2′,4′,5′-OCH_3	H	*Z*	9 ± 1.4	110 ± 1.0
6	2′,4′,6′-OCH_3	H	*E*	22 ± 3.0	10 ± 0.1
7	2′,4′,6′-CH_3	H	*E*	1600 ± 90	940 ± 80
8	2′,4′,6′-CH_2CH_3	H	*E*	11400 ± 600	2600 ± 100
9	3′,4′,5′-OCH_3	CH_3	*E*	31 ± 7.0	37 ± 2.0
Podophyllotoxin				13 ± 1.5	8 ± 0.5

[a] Data are means of three or more experiments and are reported as means ± standard error of the mean (SEM).

Table 4. Anti-proliferative activities of compounds **10–18**



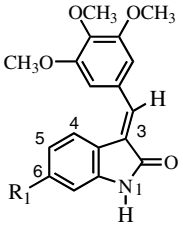
Compound	R_2	*E* or *Z* isomer	PC-3[a] (IC_{50} nM)	MDA-MB-231 [a] (IC_{50} nM)
1	3′,4′,5′-OCH_3	*E*	8 ± 2.3	0.9 ± 0.07
10	3′,5′-OCH_3	*E*	280 ± 20	90 ± 3.0
11	3′,4′-OCH_3	*E*	740 ± 18	860 ± 113
12	3′,4′-OCH_3	*Z*	410 ± 155	3100 ± 67
13	2′,3′-OCH_3	*E*	4200 ± 600	2200 ± 339
14	2′,4′-OCH_3	*E*	50 ± 7.0	84 ± 5.0
15	2′,5′-OCH_3	*E*	67 ± 12	75 ± 10
16	2′,6′-OCH_3	*E*	640 ± 9.0	180 ± 36
17	2′-OCH_3	*E*	1060 ± 172	730 ± 80
18	3′-OCH_3	*E*	1400 ± 144	800 ± 53
19	4′-OCH_3	*E*	12800 ± 840	8650 ± 840
Podophyllotoxin			13 ± 1.5	8 ± 0.5

[a] Data are means of three or more experiments and are reported as means ± standard error of the mean (SEM).

(compounds **2**, **5**, and **12**). Compound **2** is the *Z* isomeric form of compound **1** (Table 3). While compound **1** is a potent inhibitor of tubulin polymerization, compound **2** does not inhibit tubulin polymerization to any extent (Table 6). This might be due to stereochemical requirement for binding to tubulin and requires further investigation. However, compound **2** still possesses potent cytotoxicity in low nM range (Table 3). The cytotoxicity of compound **2** may result from its isomerization to compound **1** in solution. NMR analyses of compound **2** in solution (DMSO—in the presence of light between ^1H NMR analysis) showed that compound **2** gradually isomerized to compound **1** and attained equilibrium after 7 days with a ratio of compound **1**: compound **2** about 3:1. A similar ratio was also obtained if the NMR analyses begin with pure compound **1** in solution (Fig. 5). Since the degree and rate of isomerization may be solvent dependent, the isomerization studies of compounds **1** and **2** were also performed in the cell culture medium (RPMI 1640) used in the cell cytotoxicity assay. A similar result was obtained. Compound **2** slowly isomerized to compound **1** and attained a ratio of 3:1 (compound **1** to compound **2**) after 3 days (Fig. 6). Interestingly, a similar process is also observed between *cis* and *trans* isomers of CA-4.[14,30]

2.2.4. Conclusion. In summary, a series of 3-benzylidene-2-indolinone analogs have been prepared and tested for cytotoxicity on tumor cells and for inhibition of tubulin polymerization. The most potent of these analogs is **1**.

Table 5. Anti-proliferative activities of compounds **19**–**25**



Compound	R_1	*E* or *Z* isomer	PC-3[a] (IC_{50} nM)	MDA-MB-231[a] (IC_{50} nM)
1	OCH_3	*E*	8 ± 2.3	0.9 ± 0.07
20	H	*E*	>30000	9400 ± 186
21	OCH_2CH_3	*E*	7 ± 2.0	8.0 ± 2.0
22	$O(CH_2)_2CH_3$	*E*	35 ± 4.0	70 ± 6.0
23	$O(CH_2)_3CH_3$	*E*	11 ± 3.0	NT
24	$OCH(CH_3)_2$	*E*	35 ± 7.0	810 ± 110
25	$OCH_2CH(CH_3)_2$	*E*	230 ± 50	11870 ± 330
Podophyllotoxin			13 ± 1.5	8 ± 0.5

NT, not tested.
[a] Data are means of three or more experiments and are reported as means ± standard error of the mean (SEM).

Table 6. Inhibition of tubulin polymerization by selected inhibitors

Compound	PC-3[a] (IC_{50} nM)	MDA-MB-231[a] (IC_{50} nM)	Inhibition of tubulin polymerization[a] (IC_{50} μM)
1	8 ± 2.3	0.9 ± 0.07	4.5 ± 0.8
2	34 ± 10	4.0 ± 1.0	> 40
3	5800 ± 60	1100 ± 140	> 40
4	6 ± 1.5	4.0 ± 0.6	11.3 ± 1.2
5	9 ± 1.4	110 ± 1	> 40
6	22 ± 3.0	10 ± 0.1	10.3 ± 0.3
9	31 ± 7.0	37 ± 2.0	13.4 ± 1.1
10	280 ± 20	90 ± 3.0	22.2 ± 2.1
17	13000 ± 840	8650 ± 840	> 40
21	>30000	9400 ± 200	>40
Podophyllotoxin	13 ± 1.5	8.0 ± 0.5	1.2 ± 0.3

[a] Data are means of three or more experiments and are reported as means ± standard error of the mean (SEM).

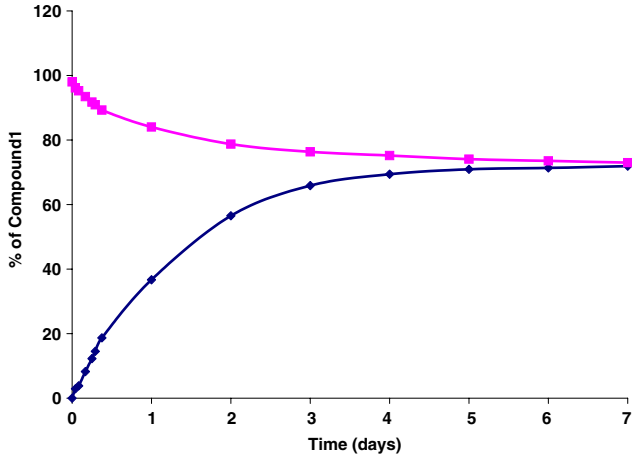


Figure 5. Isomerization study of compounds **1** (■) and **2** (♦). Pure compounds **1** and **2** are dissolved in deuterated DMSO and the rate of isomerization was measured using NMR spectroscopy. The result is presented as % compound **1** vs time (day).

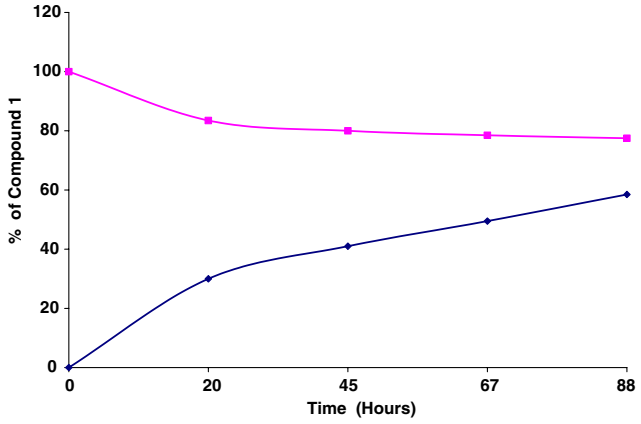


Figure 6. Isomerization of pure compounds **1** (■) and **2** (♦) in cell culture media (RPMI 1640) with time. The result is presented as % compound **1** vs time (h).

NCI 60 cell line screen showed that **1** is extremely cytotoxic with GI_{50} values below 10 nM toward 45 out of 53 human cancer cell lines tested. It caused a G_2/M phase arrest in the cell cycle following 24-h incubation. It is clear from the results that two structural features namely the 6-OCH_3 group in the indolinone ring and the trimethoxy groups in the benzylidene ring are essential for potent cytotoxicity and inhibition of tubulin polymerization. The study

contributes to the knowledge about the structure–activity-relationship of 3-benzylidene-2-indolinone analogs.

Since the compounds are derived from the KDR inhibitor SU5416, studies are currently underway to examine if the compounds also inhibit the receptor. Potentially, some of the compounds could be dual antitubulin–anti-angiogenic agents.

3. Experimental

Chemicals and biochemicals were purchased from commercial vendors and checked for purity by thin-layer chromatography and NMR before use. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra were obtained on Bruker (250 MHz) FT-NMR instrument. High-resolution mass spectra were recorded on a Micromass QTOF-Electrospray mass spectrometer at The Ohio State University Campus Chemical Instrumentation Center. Analytical HPLC was performed with reverse phase C18 column (Resolvex C18, 4.6 mm × 25 cm; Fisher Scientific, Pittsburgh, PA) using a Beckman 'System Gold' HPLC, Model 127 pump and Model 166 detector.

3.1. Synthesis of (*E*)-1,3-Dihydro-6-methoxy-3-(2,3,4-trimethoxybenzylidene)-1*H*-indol-2-one (3)

A mixture of 6-methoxy-indolin-2-one (0.15 g, 0.92 mmol), 2,3,4-trimethoxybenzaldehyde (0.36 g, 1.84 mmol), and piperidine (0.01 ml, 0.1 mmol) in ethanol (15 ml) was stirred at 80 °C for 2.5 h. The mixture was cooled to room temperature and concentrated under reduced pressure to form a yellow solid. The solid was washed with a equal mixture of hexane–ethyl acetate (50 ml) and purified by silica gel chromatography (hexane/EtOAc/MeOH, 10:10:1), yielding pure compound **3** (0.13 g, 40%): mp 181–183 °C; ^{1}H NMR (DMSO-d_6): δ 3.75 (s, 3H, –OCH_3), 3.80 (s, 3H, –OCH_3), 3.82 (s, 3H, –OCH_3), 3.88 (s, 3H, –OCH_3), 6.42 (m, 2H, Ph), 6.95 (d, J = 8.7 Hz, 1H, Ph), 7.42 (s, 1H, vinyl-H), 7.45 (m, 2H, Ph), 10.53 (s, 1H, NH); HRMS: calcd for $[C_{19}H_{19}NO_5Na]^+$: 364.11554; found: 364.11574.

3.2. (*Z*)-1,3-Dihydro-6-methoxy-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (2)

Yellow solid (28% yield): mp 229–231 °C; ^{1}H NMR (DMSO-d_6): δ 3.74 (s, 3H, –OCH_3), 3.78 (s, 3H, –OCH_3), 3.84 (s, 6H, –OCH_3), 6.41 (d, J = 2.4 Hz, 1H, Ph), 6.57 (dd, J = 8.4, 2.4 Hz, 1H, Ph), 7.57 (d, J = 8.4 Hz, 1H, Ph), 7.58 (s, 1H, vinyl-H), 7.94 (s, 2H, Ph), 10.53 (s, 1H, NH); HRMS: calcd for $[C_{19}H_{19}NO_5Na]^+$: 364.11554; found: 364.11441.

3.3. (*E*)-1,3-Dihydro-6-methoxy-3-(2,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (4)

Yellow solid (48% yield): mp 175–177 °C; ^{1}H NMR (DMSO-d_6): δ 3.71 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.91 (s, 6H, –OCH_3), 6.42 (d, J = 2.4 Hz, 1H, Ph), 6.47 (dd, J = 8.4, 2.4 Hz, 1H, Ph), 6.80 (s, 1H, Ph), 7.25 (s, 1H, Ph), 7.49 (s, 1H, vinyl-H), 7.52 (d, J = 8.4 Hz, 1H, Ph), 10.47 (s, 1H, NH); HRMS: calcd for $[C_{19}H_{19}NO_5Na]^+$: 364.11554; found: 364.11481.

3.4. (*Z*)-1,3-Dihydro-6-methoxy-3-(2,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (5)

Yellow solid (39% yield): mp 236–238 °C; ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 6H, –OCH_3), 3.88 (s, 3H, –OCH_3), 3.91 (s, 3H, –OCH_3), 6.39 (d, J = 2.4 Hz, 1H, Ph), 6.53 (dd, J = 8.4, 2.4 Hz, 1H, Ph), 6.44 (d, J = 8.4 Hz, 1H, Ph), 7.43 (d, J = 8.4 Hz, 2H, Ph), 7.77 (s, 1H, vinyl-H), 8.81 (s, 1H, Ph), 10.45 (s, 1H, NH); HRMS: calcd for $[C_{19}H_{19}NO_5Na]^+$: 364.11554; found: 364.11473.

3.5. (*E*)-1,3-Dihydro-6-methoxy-3-(2,4,6-trimethoxybenzylidene)-1*H*-indol-2-one (6)

Yellow solid (58% yield): mp 202–204 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, –OCH_3), 3.75 (s, 6H, –OCH_3), 3.87 (s, 3H, –OCH_3), 6.37 (m, 4H, Ph), 6.70 (d, J = 8.4 Hz, 1H, Ph), 7.23 (s, 1H, vinyl-H), 10.37 (s, 1H, NH); HRMS: calcd for $[C_{19}H_{19}NO_5Na]^+$: 364.11554; found: 364.11451.

3.6. (*E*)-1,3-Dihydro-6-methoxy-3-(2,4,6-trimethylbenzylidene)-1*H*-indol-2-one (7)

Yellow solid (32% yield): mp 218–220 °C; ^{1}H NMR (DMSO-d_6): δ 2.10 (s, 6H, –CH_3), 2.29 (s, 3H, –CH_3), 3.71 (s, 3H, –OCH_3), 6.33 (s, 2H, Ph), 6.39 (br s, 1H, Ph), 6.98 (br s, 2H, Ph), 7.42 (s, 1H, vinyl-H), 10.53 (s, 1H, NH); HRMS: calcd for $[C_{19}H_{19}NO_2Na]^+$: 316.13080; found: 316.13217.

3.7. (*E*)-1,3-Dihydro-6-methoxy-3-(2,4,6-triethylbenzylidene)-1*H*-indol-2-one (8)

Yellow solid (28% yield): mp 164–166 °C; ^{1}H NMR (DMSO-d_6): δ 0.99 (t, J = 7.5 Hz, 6H, –CH_3), 1.23 (t, J = 7.5 Hz, 3H, –CH_3), 2.44 (m, 4H, –CH_2), 2.60 (q, J = 7.5 Hz, 2H, –CH_2), 3.70 (s, 3H, –OCH_3), 6.27 (m, 2H, Ph), 6.38 (d, J = 2.1 Hz, 1H, Ph), 7.03 (s, 2H, Ph), 7.52 (s, 1H, vinyl-H), 10.53 (s, 1H, NH); HRMS: calcd for $[C_{22}H_{25}NO_2Na]^+$: 358.17775; found: 358.17904.

3.8. (*E*)-1,3-Dihydro-6-methoxy-1-methyl-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (9)

Yellow solid (42% yield): mp 252–254 °C; ^{1}H NMR ($CDCl_3$): δ 3.27 (s, 3H, –CH_3), 3.85 (s, 3H, –OCH_3), 3.88 (s, 6H, –OCH_3), 3.93 (s, 6H, –OCH_3), 6.43 (m, 2H, Ph), 6.89 (s, 2H, Ph), 7.62 (s, 1H, vinyl-H), 7.71 (d, J = 8.1 Hz, 1H, Ph), HRMS: calcd for $[C_{20}H_{21}NO_5Na]^+$: 378.13119; found: 378.13202.

3.9. (*E*)-1,3-Dihydro-6-methoxy-3-(3,5-dimethoxybenzylidene)-1*H*-indol-2-one (10)

Yellow solid: mp 156–158 °C; ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 3H, –OCH_3), 3.78 (s, 6H, –OCH_3), 6.42

(d, $J = 2.4$ Hz, 1H, Ph), 6.48 (dd, $J = 8.4$, 2.4 Hz, 1H, Ph), 6.58 (t, $J = 2.4$ Hz, 1H, Ph), 6.82 (d, $J = 2.4$ Hz, 2H, Ph), 7.38 (s, 1H, vinyl-H), 7.53 (d, $J = 8.4$ Hz, 1H, Ph), 10.55 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10575.

3.10. (*E*)-1,3-Dihydro-6-methoxy-3-(3,4-dimethoxybenzylidene)-1*H*-indol-2-one (11)

Yellow solid (39% yield): mp 184–186 °C; ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 3H, –OCH_3), 3.80 (s, 3H, –OCH_3), 3.84 (s, 3H, –OCH_3), 6.45 (m, 2H, Ph), 7.10 (d, $J = 8.4$ Hz, 1H, Ph), 7.31 (m, 2H, Ph), 7.40 (s, 1H, vinyl-H), 7.65 (d, $J = 2.4$ Hz, 1H, Ph), 10.51 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10575.

3.11. (*Z*)-1,3-Dihydro-6-methoxy-3-(3,4-dimethoxybenzylidene)-1*H*-indol-2-one (12)

Yellow solid (25% yield): mp 252–254 °C; ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 3H, –OCH_3), 3.82 (s, 6H, –OCH_3), 6.40 (d, $J = 2.4$ Hz, 1H, Ph), 6.55 (dd, $J = 8.4$, 2.4 Hz, 1H, Ph), 7.04 (d, $J = 8.4$ Hz, 1H, Ph), 7.57 (d, $J = 8.4$ Hz, 1H, Ph), 7.57 (s, 1H, vinyl-H), 7.77 (dd, $J = 8.4$, 2.4 Hz, 1H, Ph), 8.59 (d, $J = 2.4$ Hz, 1H, Ph), 10.50 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10405.

3.12. (*E*)-1,3-Dihydro-6-methoxy-3-(2,3-dimethoxybenzylidene)-1*H*-indol-2-one (13)

Yellow solid (37% yield): mp 172–173 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.86 (s, 3H, –OCH_3), 6.41 (m, 2H, Ph), 7.20 (m, 3H, Ph), 7.30 (d, $J = 8.4$ Hz, 1H, Ph), 7.46 (s, 1H, vinyl-H), 10.56 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10575.

3.13. (*E*)-1,3-Dihydro-6-methoxy-3-(2,4-dimethoxybenzylidene)-1*H*-indol-2-one (14)

Yellow solid (50% yield): mp 198–199 °C; ^{1}H NMR (DMSO-d_6): δ 3.75 (s, 3H, –OCH_3), 3.86 (s, 6H, –OCH_3), 6.43 (m, 2H, Ph), 6.67 (m, 2H, Ph), 7.43 (m, 2H, Ph), 7.65 (d, $J = 8.7$ Hz, 1H, Ph), 10.48 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10573.

3.14. (*E*)-1,3-Dihydro-6-methoxy-3-(2,5-dimethoxybenzylidene)-1*H*-indol-2-one (15)

Yellow solid (65% yield): mp 179–180 °C; ^{1}H NMR (DMSO-d_6):™ 3.73 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3), 3.79 (s, 3H, –OCH_3), 6.44 (m, 2H, Ph), 7.05 (m, 2H, Ph), 7.20 (d, $J = 2.7$ Hz, 1H, Ph), 7.39 (d, $J = 8.4$ Hz, 1H, Ph), 7.44 (s, 1H, vinyl-H), 10.54 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10460.

3.15. (*E*)-1,3-Dihydro-6-methoxy-3-(2,6-dimethoxybenzylidene)-1*H*-indol-2-one (16)

Yellow solid (68% yield): mp 215–217 °C; ^{1}H NMR (DMSO-d_6): δ 3.73 (s, 3H, –OCH_3), 3.74 (s, 6H, –OCH_3), 6.38 (m, 2H, Ph), 6.60 (m, 1H, Ph), 6.76 (d, $J = 8.4$ Hz, 2H, Ph), 7.25 (s, 1H, vinyl-H), 7.41 (t, $J = 8.4$ Hz, 1H, Ph), 10.43 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10509.

3.16. (*E*)-1,3-Dihydro-6-methoxy-3-(2-methoxybenzylidene)-1*H*-indol-2-one (17)

Yellow solid (65% yield): mp 208–209 °C; ^{1}H NMR (DMSO-d_6): δ 3.75 (s, 3H, –OCH_3), 3.85 (s, 3H, –OCH_3), 6.40 (m, 2H, Ph), 7.06 (t, $J = 8.1$ Hz, 1H, Ph), 7.14 (d, $J = 8.1$ Hz, 1H, Ph), 7.33 (d, $J = 8.1$ Hz, 1H, Ph), 7.44 (d, $J = 8.1$ Hz, 1H, Ph), 7.45 (s, 1H, vinyl-H), 7.65 (d, $J = 8.1$ Hz, 1H, Ph), 10.54 (s, 1H, NH); HRMS: calcd for $[C_{17}H_{15}NO_3Na]^+$: 304.09441; found: 304.09430.

3.17. (*E*)-1,3-Dihydro-6-methoxy-3-(3-methoxybenzylidene)-1*H*-indol-2-one (18)

Yellow solid (69% yield): mp 162–163 °C; ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 3H, –OCH_3), 3.80 (s, 3H, –OCH_3), 6.44 (m, 2H, Ph), 7.02 (d, $J = 8.4$ Hz, 1H, Ph), 7.24 (m, 2H, Ph), 7.42 (s, 1H, vinyl-H), 7.43 (t, $J = 8.4$ Hz, 1H, Ph), 7.50 (d, $J = 8.4$ Hz, 1H, Ph), 10.56 (s, 1H, NH); HRMS: calcd for $[C_{17}H_{15}NO_3Na]^+$: 304.09441; found: 304.09513.

3.18. (*E*)-1,3-Dihydro-6-methoxy-3-(4-methoxybenzylidene)-1*H*-indol-2-one (19)

Yellow solid (77% yield): mp 180–181 °C; ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 3H, –OCH_3), 3.84 (s, 3H, –OCH_3), 6.45 (m, 2H, Ph), 7.07 (d, $J = 8.4$ Hz, 2H, Ph), 7.41 (s, 1H, vinyl-H), 7.57 (d, $J = 8.4$ Hz, 1H, Ph), 7.67 (d, $J = 8.4$ Hz, 2H, Ph), 10.51 (s, 1H, NH); HRMS: calcd for $[C_{17}H_{15}NO_3Na]^+$: 304.09441; found: 304.09511.

3.19. (*E*)-1,3-Dihydro-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (20)

Yellow solid (61% yield): mp 189–191 °C; ^{1}H NMR (DMSO-d_6): δ 3.75 (s, 3H, –OCH_3), 3.85 (s, 6H, –OCH_3), 6.84 (d, $J = 7.5$ Hz, 1H, Ph), 7.00 (t, $J = 7.5$ Hz, 1H, Ph), 7.21 (t, $J = 7.5$ Hz, 1H, Ph), 7.68 (d, $J = 7.5$ Hz, 1H, Ph), 7.77 (s, 1H, vinyl-H), 8.02 (s, 2H, Ph), 10.59 (s, 1H, NH); HRMS: calcd for $[C_{18}H_{17}NO_4Na]^+$: 334.10498; found: 334.10575.

3.20. (*E*)-1,3-Dihydro-6-ethoxy-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (21)

Yellow solid (60% yield): mp 169–170 °C; ^{1}H NMR (DMSO-d_6): δ 1.32 (t, $J = 6.9$ Hz, 3H, –CH_3), 3.74 (s, 3H, –OCH_3), 3.82 (s, 6H, –OCH_3), 4.02 (q, $J = 6.9$ Hz, 2H, –OCH_2), 6.40 (d, $J = 2.1$ Hz, 1H, Ph), 6.46 (d, $J = 8.4$ Hz, 1H, Ph), 7.02 (s, 2H, Ph), 7.38 (s, 1H, Ph), 7.63 (d, $J = 8.4$ Hz, 1H, Ph), 10.48 (s, 1H, NH); HRMS: calcd for $[C_{20}H_{21}NO_5Na]^+$: 378.13119; found: 378.13103.

3.21. (*E*)-1,3-Dihydro-6-propoxy-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (22)

Yellow solid (62% yield): mp 142–143 °C; ^{1}H NMR (DMSO-d_6): δ 1.02 (t, J = 6.7 Hz, 3H, –CH_3), 1.78 (m, 2H, –CH_2), 3.74 (s, 3H, –OCH_3), 3.82 (s, 6H, –OCH_3), 3.95 (t, J = 6.9 Hz, 2H, –OCH_2), 6.41 (d, J = 2.1 Hz, 1H, Ph), 6.48 (dd, J = 8.6 Hz, 2.1 Hz, 1H, Ph), 7.03 (s, 2H, Ph), 7.38 (s, 1H, Ph), 7.64 (d, J = 8.6 Hz, 1H, Ph), 10.51 (s, 1H, NH); HRMS: calcd for $[C_{21}H_{23}NO_5Na]^+$: 392.146842; found: 392.14629.

3.22. (*E*)-1,3-Dihydro-6-butoxy-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (23)

Yellow solid (65% yield): mp 182–183.5 °C; ^{1}H NMR (DMSO-d_6): δ 0.91 (t, J = 7.2 Hz, 3H, –CH_3), 1.41 (m, 2H, –CH_2), 1.68 (m, 2H, –CH_2), 3.73 (s, 3H, –OCH_3), 3.80 (s, 6H, –OCH_3), 3.96 (m, 2H, –OCH_2), 6.41 (d, J = 2.1 Hz, 1H, Ph), 6.49 (d, J = 7.8 Hz, 1H, Ph), 7.02 (s, 2H, Ph), 7.38 (s, 1H, Ph), 7.64 (d, J = 7.8 Hz, 1H, Ph), 10.53 (s, 1H, NH); HRMS: calcd for $[C_{22}H_{25}NO_5Na]^+$: 406.16249; found: 406.16119.

3.23. (*E*)-1,3-Dihydro-6-isopropoxy-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (24)

Yellow solid (62% yield): mp 187.5–189 °C; ^{1}H NMR (DMSO-d_6): δ 1.26 (d, J = 6.0 Hz, 6H, –$(CH_3)_2$), 3.74 (s, 3H, –OCH_3), 3.82 (s, 6H, –OCH_3), 4.59 (m, 1H, –OCH), 6.40 (d, J = 2.1 Hz, 1H, Ph), 6.46 (d, J = 8.4 Hz, 1H, Ph), 7.02 (s, 2H, Ph), 7.38 (s, 1H, Ph), 7.64 (d, J = 8.4 Hz, 1H, Ph), 10.48 (s, 1H, NH); HRMS: calcd for $[C_{21}H_{23}NO_5Na]^+$: 392.146842; found: 392.14528.

3.24. (*E*)-1,3-Dihydro-6-isobutoxy-3-(3,4,5-trimethoxybenzylidene)-1*H*-indol-2-one (25)

Yellow solid (57% yield): mp 107–109 °C; ^{1}H NMR (DMSO-d_6): δ 0.97 (d, J = 6.6 Hz, 6H, –$(CH_3)_2$), 1.24 (m, 1H, –CH), 3.74 (s, 3H, –OCH_3), 3.83 (s, 6H, –OCH_3), 3.96 (d, J = 6.3 Hz, 2H, –OCH_2), 6.41 (d, J = 2.1 Hz, 1H, Ph), 6.49 (dd, J = 8.4 Hz, 2.1 Hz, 1H, Ph), 7.03 (s, 2H, Ph), 7.38 (s, 1H, Ph), 7.65 (d, J = 8.4 Hz, 1H, Ph), 10.52 (s, 1H, NH); HRMS: calcd for $[C_{22}H_{25}NO_5Na]^+$: 406.16249; found: 406.16233.

3.25. Cell lines and culture

Tumor cell lines (MDA-MB-231; prostate: PC-3) were originally obtained from American type culture collection. PC-3 cells were cultured in complete RPMI 1640 (Gibco, Invitrogen, Grand Island, NY) + 10% FBS (GIBCO), 2 mM L-glutamine (GIBCO), and 1% gentamicin (GIBCO). MDA-MB-231 cells were cultured in DMEM F-12 + 10% FBS, 2 mM L-glutamine, and 1% gentamicin.

3.26. Cell growth inhibition assay

Cells were grown in suspension cultures at 37 °C in a humidified 5% CO_2 atmosphere. Cells were plated into 96-well plates at a cell density of 1000 cells per well in 100 μl of media and were allowed to attach overnight. Varying concentrations of compounds were added to the cells with a final volume per well of 200 μl. The treated cells were incubated under 37 °C, 5% CO_2 for 72 h. Cell viability was determined by Promega CellTiter 96 Aqueous Non-Radioactive Cell Proliferation assay (MTS). IC_{50} values were calculated using the SOFTmax Pro plate reader program. Each compound was tested at least three times in triplicate.

3.27. Tubulin assembly assay

Porcine brain tubulin was purified according to the reported procedure.[31,32] The assay was carried out on a 96-well half area (Costar) plate in PME buffer [0.1 M PIPES (pH 6.9), 1 mM $MgCl_2$, and 1 mM EGTA] at a protein concentration of 1.5 mg/ml (15 mM). The components of disassembly assay included PME buffer, various drug dilutions, DMSO (final concentration 10% v/v) and tubulin were added to 96-well half-area plate kept on ice. Reaction was initiated by simultaneous addition of GTP to a 1 mM final concentration to all of the wells. Polymerization was monitored by the increase/change in absorbance at 351 nm using a Spectra Plus micro plate reader. Podophyllotoxin was used as a positive control in each assay.

3.28. Isomerization studies of compounds 1 and 2

(a) Isomerization study using ^{1}H NMR spectroscopy—pure compounds **1** and **2** (3–4 mg) were dissolved separately in DMSO-d_6 and placed in NMR tubes. NMR spectra were then obtained in 1, 2, 4, 8, 12, 24 h and then once daily for up to 10 days. The extent of isomerization was measured by comparing the peak ratio of the vinyl protons of compound **1** (7.40 ppm) and compound **2** (7.58 ppm). The results are plotted as % compound **1** versus time (day).

(b) Isomerization study of compounds **1** and **2** in cell culture media—compounds **1** and **2** (2.3 mg of compound **1** and 2.5 mg of compound **2**) were each dissolved in 3 ml of DMSO. An aliquot (2.3 ml) of the DMSO solutions (compounds **1** and **2**) were added separately to RPMI media (19.77 ml). The concentrations of compounds **1** and **2** in the media were 2.58×10^{-5} and 2.8×10^{-5}M, respectively. The concentrations were chosen due to the detection limit of the UV detector. The media containing the compounds were placed in a 24-well plate (2 ml per well). The plate was placed in an incubator (37 °C, 5% CO_2). At set time intervals (0, 20, 45, 67, and 88 h) media were removed for HPLC analysis. The media in each well (2 wells per time interval per compound) were extracted with CH_2Cl_2 (2× 1 ml). An analytical sample (10 μl) of the CH_2Cl_2 layer was injected into HPLC to determine the degree of isomerization. HPLC grade water and methanol (3:7) were used with a flow rate of 1 ml/min applied with UV detection at 352 nm.

Comparison of compound **1** to **2** absorption peaks showed percentage of each compound. The results are plotted as % compound **1** versus time (hour).

Acknowledgment

This work is supported in part by The Intramural Research Program of National Institute of Child Health and Human Development, NIH.

References and notes

1. Hamel, E. *Med. Res. Rev.* **1996**, *16*, 207.
2. Fahy, J.; Hill, B. T. *Curr. Pharm. Des.* **2001**, *7*, 1297.
3. Nam, N. H. *Curr. Med. Chem.* **2003**, *10*, 1697.
4. Hsieh, H. P.; Liou, J. P.; Mahindroo, N. *Curr. Pharm. Des.* **2005**, *11*, 1655.
5. Tozer, G. M.; Prise, V. E.; Wilson, J.; Locke, R. J.; Vojnovic, B.; Stratford, M. R.; Dennis, M. F.; Chaplin, D. J. *Cancer Res.* **1999**, *59*, 1626.
6. Tozer, G. M.; Kanthou, C.; Parkins, C. S.; Hill, S. A. *Int. J. Exp. Pathol.* **2002**, *83*, 21.
7. Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829.
8. Grosios, K.; Holwell, S. E.; McGown, A. T.; Pettit, G. R.; Bibby, M. C. *Br. J. Cancer* **1999**, *81*, 1318.
9. Dowlati, A.; Robertson, K.; Cooney, M.; Petros, W. P.; Stratford, M.; Jesberger, J.; Rafie, N.; Overmoyer, B.; Makkar, V.; Stambler, B.; Taylor, A.; Waas, J.; Lewin, J. S.; McCrae, K. R.; Remick, S. C. *Cancer Res.* **2002**, *62*, 3408.
10. Stevenson, J. P.; Rosen, M.; Sun, W.; Gallagher, M.; Haller, D. G.; Vaughn, D.; Giantonio, B.; Zimmer, R.; Petros, W. P.; Stratford, M.; Chaplin, D.; Young, S. L.; Schnall, M.; O'Dwyer, P. J. *J. Clin. Oncol.* **2003**, *21*, 4428.
11. Kirwan, I. G.; Loadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R.; Lippert, J. W., 3rd; Shnyder, S. D.; Cooper, P. A.; Bibby, M. C. *Clin. Cancer Res.* **2004**, *10*, 1446.
12. Galbraith, S. M.; Maxwell, R. J.; Lodge, M. A.; Tozer, G. M.; Wilson, J.; Taylor, N. J.; Stirling, J. J.; Sena, L.; Padhani, A. R.; Rustin, G. J. *J. Clin. Oncol.* **2003**, *21*, 2831.
13. LeBlanc, R.; Dickson, J.; Brown, T.; Stewart, M.; Pati, H. N.; VanDerveer, D.; Arman, H.; Harris, J.; Pennington, W.; Holt, H. L.; Lee, M. *Bioorg. Med. Chem.* **2005**, *13*, 6025.
14. Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200.
15. Ohsumi, K.; Hatanaka, T.; Fujita, K.; Nakagawa, R.; Fukuda, Y.; Nihei, Y.; Suga, Y.; Morinaga, Y.; Akiyama, Y.; Tsuji, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3153.
16. Wang, L.; Woods, K. W.; Li, Q.; Barr, K. J.; McCroskey, R. W.; Hannick, S. M.; Gherke, L.; Credo, R. B.; Hui, Y. H.; Marsh, K.; Warner, R.; Lee, J. Y.; Zielinski-Mozng, N.; Frost, D.; Rosenberg, S. H.; Sham, H. L. *J. Med. Chem.* **2002**, *45*, 1697.
17. Pettit, G. R.; Rhodes, M. R.; Herald, D. L.; Hamel, E.; Schmidt, J. M.; Pettit, R. K. *J. Med. Chem.* **2005**, *48*, 4087.
18. Pettit, G. R.; Grealish, M. P.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2000**, *43*, 2731.
19. Medarde, M.; Ramos, A. C.; Caballero, E.; Pelaez-Lamamie de Clairac, R.; Lopez, J. L.; Gravalos, D. G.; Feliciano, A. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2303.
20. Nam, N. H.; Kim, Y.; You, Y. J.; Hong, D. H.; Kim, H. M.; Ahn, B. Z. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3073.
21. Nam, N. H.; Kim, Y.; You, Y. J.; Hong, D. H.; Kim, H. M.; Ahn, B. Z. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1955.
22. Kuo, C. C.; Hsieh, H. P.; Pan, W. Y.; Chen, C. P.; Liou, J. P.; Lee, S. J.; Chang, Y. L.; Chen, L. T.; Chen, C. T.; Chang, J. Y. *Cancer Res.* **2004**, *64*, 4621.
23. Borrel, C.; Thoret, S.; Cachet, X.; Guenard, D.; Tillequin, F.; Koch, M.; Michel, S. *Bioorg. Med. Chem.* **2005**, *13*, 3853.
24. Ouyang, X.; Piatnitski, E. L.; Pattaropong, V.; Chen, X.; He, H. Y.; Kiselyov, A. S.; Velankar, A.; Kawakami, J.; Labelle, M.; Smith, L., 2nd; Lohman, J.; Lee, S. P.; Malikzay, A.; Fleming, J.; Gerlak, J.; Wang, Y.; Rosler, R. L.; Zhou, K.; Mitelman, S.; Camara, M.; Surguladze, D.; Doody, J. F.; Tuma, M. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1191.
25. Sun, L.; Tran, N.; Tang, F.; App, H.; Hirth, P.; McMahon, G.; Tang, C. *J. Med. Chem.* **1998**, *41*, 2588.
26. Sun, L.; Tran, N.; Liang, C.; Tang, F.; Rice, A.; Schreck, R.; Waltz, K.; Shawver, L. K.; McMahon, G.; Tang, C. *J. Med. Chem.* **1999**, *42*, 5120.
27. Li, P. K.; Xiao, Z.; Hu, Z.; Pandit, B.; Sun, Y.; Sackett, D. L.; Werbovetz, K.; Lewis, A.; Johnsamuel, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5382.
28. Quallich, G.; Morrissey, P. *Synthesis* **1993**, 51.
29. Moiseev, V. V.; Zalukaev, L. P. *Izvestiya Vysshikh Uchebnykh Zavedenii Kihmiya i Khimicheskaya Tekhnologiya* **1967**, *10*, 945.
30. Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1991**, *34*, 2579–2588.
31. Hamel, E.; Lin, C. M. *Biochemistry* **1984**, *23*, 4173.
32. Sackett, D. L.; Knipling, L.; Wolff, J. *Protein Expr. Purif.* **1991**, *2*, 390.